

Molecular Biology

IDENTIFICATION AND CHARACTERIZATION OF AlgZ DEPENDENT GENES  
INVOLVED IN TWITCHING MOTILITY USING A TRANSPOSABLE PROMOTER  
PROBE

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*Pseudomonas aeruginosa* is a rod-shaped, Gram negative bacterium commonly found in soil and water. In order to colonize its host and move in environments with low water content, *P.aeruginosa* utilizes a flagella-independent form of translocation known as twitching motility (TM). TM is dependent upon the extension and retraction of type IV pili. AlgZ is a DNA-binding protein previously shown to regulate the alginate biosynthetic operon. Recent research has identified *algZ* as required for TM. The role of AlgZ in TM is not due to the regulation of alginate since deletion of this operon results in a strain that still twitches. These data suggest AlgZ is regulating other genes involved in TM by activating a gene(s) required for twitching or by repressing a gene(s) which negatively regulates TM. The purpose of this study was to identify and characterize genes that are AlgZ- dependent and involved in TM. Transposon (Tn) mutagenesis was used to generate a library of insertion mutants using Tn5-pOT182 and Tn5-B22 in a *P.aeruginosa* strain containing an arabinose inducible *algZ*. Using these transposons 5,000 mutants were constructed and, 100 Tn5-pOT182 and 100 Tn5-B22 integrates were screened utilizing a 96-well Beta-galactosidase assay. Only tetracycline resistant isolates with differential expression of the reporter gene, *lacZ* with and without *algZ* induction were investigated further. Three Tn mutants with differential expression were isolated and the DNA surrounding the Tn will be cloned and sequenced. These genes will then be characterized further with regard to *algZ* dependence and their role in TM.